

AP Biology

Unit 3.1 – DNA Structure, Function, Expression & Manipulation

Notes & Practice Quiz

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SECTION 1 – NUCLEIC ACID STRUCTURE

1.1 – WHAT ARE NUCLEIC ACIDS COMPOSED OF?

Nucleic Acids are polymers of nucleotides used to store & transmit hereditary information. The two nucleic acids are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). The **nucleotide** monomers are made of 3 parts: A 5-carbon sugar, a phosphate group and a nitrogen base. The 5-carbon sugar is **deoxyribose** in DNA and **ribose** in RNA, giving each nucleic acid its respective name. The nitrogen bases are classified by how many connected rings of nitrogen/carbon atoms there are. One ring of atoms forms nitrogen bases called **pyrimidines**. 2 rings of atoms form nitrogen bases called **purines**. Draw figure 5.26 b & c on p.87 of the nucleotide general structure, the sugars & nitrogen bases.

1.2 – HOW DO NUCLEOTIDES BOND TOGETHER TO FORM A NUCLEIC ACID?

Draw figure 16.5 on p.308. A nucleotide has a specific directionality to it, termed the **5 prime (5') end** and the **3 prime (3') end**. The numbers refer to the position of the carbon atoms in the sugar. The phosphate group is attached to the 5' carbon and the 3' carbon has an oxygen-hydrogen called a **hydroxyl group** (OH group). When nucleotides are linked together, the phosphate group of a new nucleotide always adds onto the 3' OH group of another nucleotide. The reason for this is that each free nucleotide is actually a high-energy triphosphate nucleotide (ATP, GTP, CTP, TTP, UTP) that releases energy for its own bonding to the next nucleotide like ATP does when it is hydrolyzed. Figure 16.5 shows the sugars & phosphates linked together in a DNA strand; this is called the **sugar-phosphate backbone** of a nucleic acid. The nitrogen bases are not involved in elongating a single nucleic acid strand, but do function when 2 different strands of nucleic acid bond together.

1.3 – HOW DO 2 NUCLEIC ACID STRANDS BOND TOGETHER?

The rules of **complementary base-pairing** state that in DNA: the bases Adenine (A) and Thymine (T) bond together & the bases Guanine (G) and Cytosine (C) bond together. In RNA: the bases Adenine (A) and Uracil (U) bond together & the bases Guanine (G) and Cytosine (C) bond together. The reason underlying these rules is that when the bases bond together, it is by **hydrogen bonding** like in water and the most stable (*complementary*) formations are the 3 hydrogen bonds forming between G-C and the 2 hydrogen bonds forming between A-T & A-U. When 2 nucleic acid strands bond together at their bases, the hydrogen bonds are just weak enough so that the 2 strands can be separated by enzymes when needed but strong enough to hold 2 strands together. Draw figure 16.7 b on p.309 of the DNA hydrogen bonding.

1.4 – HOW ARE NUCLEIC ACID STRANDS ORIENTED WHEN BONDED?

As mentioned in section 1.2, there is directionality to nucleotides and this directionality also leads to directionality of the nucleic acid polymers. The 5' end of a nucleic is the end with the phosphate group and the 3' end is the end with the hydroxyl group. Because bonding of nucleic acids occurs through their bases, the 2 strands always run in opposite directions like a road with cars going in opposite directions. This opposite-running orientation between 2 nucleic acid strands is called **antiparallel** directionality. This is also demonstrated in figure 16.7 b on p.309. 2 DNA strands bond antiparallel in the double helix (twisted-ladder shape), 1 DNA strand and 1 mRNA strand bond antiparallel during transcription, mRNA codons and tRNA anticodons bond antiparallel during translation, and RNA can even twist & fold to bond with itself antiparallel.

SECTION 2 – DNA REPLICATION

2.1 – WHAT IS THE PURPOSE OF DNA REPLICATION?

DNA serves as the genetic instructions inside of all cells. With few exceptions, each cell of multicellular organisms contains the same DNA (outside of mutations). When cells divide they are achieving growth, repair or production of reproductive cells. However, before a cell divides it must **replicate** its DNA so that each new cell will have a copy of the genetic material.

2.2 – HOW IS DNA'S STRUCTURE SUITED FOR ITS REPLICATION?

When the hydrogen bonding between parts of 2 DNA strands is undone by enzymes, it un-pairs the bases. This leaves them with a strong affinity to re-bond with their complementary bases. Instead of re-bonding with the other strand, individual free nucleotides in the nucleus will be added to both original DNA strands. In this manner, each original DNA strand is called a **template strand** (or parental) for new nucleotides to be bonded to. This mechanism of adding new nucleotides to an existing DNA strand is called the **semiconservative replication model**, since half of each completely replicated DNA molecule will be original DNA and the other half will be new DNA.

2.3 – HOW DOES DNA REPLICATION BEGIN?

DNA has sequences of bases called **origins of replication** that are recognized by the first enzyme called helicase. Prokaryotes have only single, small circular chromosomes and need only 1 origin of replication. Eukaryotes have multiple, long linear chromosomes and have many origins of replication per chromosome. The replication steps to be discussed are based on bacteria models but eukaryote replication is assumed to be very similar. As **helicase** recognizes & unwinds DNA, it creates a **replication "bubble"** that has the bases separated within the bubble. Single-stranded binding proteins keep the 2 template strands from re-bonding together. The points where the bubble meets the unopened DNA regions are called **replication forks**. Draw figure 16.12 on p.313 of these topics.

2.4 – HOW DOES REPLICATION OF THE LEADING STRANDS OCCUR?

The enzyme **primase** begins the replication process by bonding a short RNA sequence called a **primer** that DNA nucleotides can then be added onto. A primer is added to the **center** of each template strand in the bubble. Next **DNA polymerase** enzymes will begin to continuously add complementary nucleotides towards the direction of the replication forks. Recall that new nucleotides are only added to the 3' end of a nucleotide strand for the triphosphate energy release purpose. For this reason, replication always occurs in a 5' → 3' direction, where the 3' end is always elongating from new nucleotides being added at that location. Draw figure 16.15 on p.315 of replication in the bubble.

As nucleotides are added towards the replication fork, helicase will continue to unwind DNA and the bubble will expand horizontally. Because replicating towards the replication forks always exposes the 3' ends for nucleotide additions, this growth is called **continuous** and the strands growing in this direction are called **leading strands**.

2.5 – HOW DOES REPLICATION OF THE LAGGING STRANDS OCCUR?

Adding nucleotides away from the replication fork is a bit trickier because the leading strand primer was added at the center of each side of the replication bubble template strand and then the leading strand grew away from the center, towards the replication fork. What about the other halves of the bubble that still have exposed template DNA? The 5' end of the primer cannot be added onto; so instead, another RNA primer will be added right at the opposite end's replication fork and added onto back towards the leading strand's primer, still in the 5' → 3' direction. When the replication bubble opens

more, another primer will be added followed by more nucleotide additions. Because replicating away from the replication forks requires many primers resulting in DNA being made essentially in fragments, this growth is called **discontinuous** and the strands growing in this direction are called **lagging strands**. Draw figure 16.16 on p.316 of how the lagging strand grows.

2.6 – HOW IS REPLICATION COMPLETED, CHECKED & REPAIRED?

Once all of the DNA has been replicated, the leading strand & lagging strand primers must be excised & replaced with DNA by DNA polymerase. Finally, all of the fragments must be joined by the enzyme ligase. Interestingly, the ends of all lagging strands are incapable of replacing the final primer on eukaryote DNA because there is no 3' end! The result of this is that all of a eukaryote's chromosomes slowly shrink because the lagging strands can never fully be replicated. It turns out that the very ends of all our chromosomes are actually non-coding DNA sequences called **telomeres** that don't affect us when they are deleted. Interestingly there is a limit of how long our telomeres are and when they are all used up, this begins to affect us and is closely linked to aging and overall life span.

Proofreading also occurs during replication by DNA polymerase right after it adds each nucleotide; however, this process occurs so rapidly that mistakes are made. Most times they are simple mismatches (A with C or T with G) but other large-scale errors also occur and sometimes not during the replication process. Some can be detected and fixed but other times they remain forever and are by definition **mutations**.

SECTION 3 – GENE EXPRESSION 1: TRANSCRIPTION

3.1 – WHAT IS THE FUNCTION OF GENE EXPRESSION?

DNA is the coded information for all of an organism's traits. Most of these traits are based on or controlled by proteins. A **gene** is a sequence of nucleotides on a chromosome that will be used to produce a protein. In simple organisms and simple human traits a single gene codes for a single protein. However, many complex traits and even single proteins require multiple genes. The process of protein synthesis, or **gene expression**, starts by reading DNA, forming a complementary mRNA (**transcription**) and finally forming a sequence of amino acids from the mRNA (**translation**) that will become a protein with a distinct phenotype.

3.2 – WHAT DETERMINES WHICH GENES ARE EXPRESSED?

Recall that during development each cell has a unique set of proteins from asymmetric divisions. These proteins that influence the expression of DNA throughout an organism's lifetime are called **transcription factors**. One molecule of DNA (a chromosome) can have thousands of genes but they will only be expressed if there are matching transcription factors to recognize the genes & initiate transcription. This specific requirement makes differential gene expression possible and thus specialized cells based on their specific phenotypes expressed. For example, the long cell projections called axons present on only nerve cells are expressed because only nerve cells have transcription factors to express genes that code for those axons.

3.3 – HOW IS TRANSCRIPTION INITIATED?

A gene has an initiation sequence called a **promoter**. At the beginning of the promoter sequence is a sequence of repeating T's and A's called the **TATA box** & is the region where transcription factors bind to. Once bound, the transcription factors attract the enzyme **RNA polymerase** that will begin transcribing the DNA into mRNA by complementary base pairing. Remember though that *RNA has U in place of T*, so if a DNA sequence is GGATTCT, then the transcribed mRNA will be CCUAAGA.

3.4 – HOW DOES DIRECTIONALITY PLAY A ROLE IN TRANSCRIPTION?

Recall that the polymerases only add new nucleotides onto the 3' end of a growing nucleic acid sequence. DNA is double stranded and each strand has a specific direction. For any gene it will always be on one of the two strands but it could be running in the 3' → 5' direction or in the 5' → 3' direction. Transcription of a required gene will always occur so that RNA polymerase can build an mRNA in the 5' → 3' direction. Draw figure 17.4 on p.329 before proceeding and then continue reading these notes.

RNA polymerase will read the DNA **template strand** as a means to build the mRNA by base-pairing. Notice that in figure 17.4 the mRNA sequence is 5'-UGGUUUGGCUCA-3' which is complementary & antiparallel to the DNA template sequence 3'-ACCAAACCGAGT-5'. The other pattern to notice is that the other DNA strand has the same code & directionality as the mRNA except T's are replaced with U's. This DNA strand that is the coded message is called the DNA **coding strand**.

3.5 – HOW IS TRANSCRIPTION TERMINATED?

Prokaryotes have a sequence called the terminator that immediately stops transcription. The mRNA is then complete and then translation on a ribosome occurs, sometimes occurring while transcription is still happening. Eukaryotes have a sequence called the polyadenylation sequence that signals transcription will end about 10-35 nucleotides later. The mRNA will then detach and undergo post-transcriptional modifications.

SECTION 4 – GENE EXPRESSION 2: TRANSLATION

4.1 – HOW IS EUKARYOTE mRNA MODIFIED BEFORE TRANSLATION?

The initial mRNA transcript formed is called pre-mRNA and must undergo several modifications before translation. First the 5' end will have a GTP molecule attached called 5' cap. Second, the 3' end will have a stretch of A's added called a poly-A tail. Both of these modifications assist the mRNA's transit from the nucleus to the cytoplasm where it will attach to a ribosome. The third and most intriguing step is that the mRNA will have non-coding sequences called **introns** excised (cut) out and the coding sequences called **exons** spliced (pasted) together by a protein/RNA complex called a **spliceosome**. This process is called **RNA splicing**. Remember that many genes can be on a DNA molecule and many times they actually overlap so one gene's exons may be another gene's introns. This allows the same exact DNA molecule to be transcribed but excised/spliced differently, called **alternative splicing**. The mature mRNA transcript will then exit the nucleus and attach to a ribosome where translation begins. These eukaryote modifications are diagramed on pp.334—335, draw them in your notes for reference.

4.2 – HOW IS TRANSLATION INITIATED & A READING FRAME ESTABLISHED?

Translation is initiated when the 5' cap attaches to a ribosome. The ribosome then begins reading the mRNA sequence until it reaches the "Start" signal. Ribosomes have a very specific reading frame based on a **triplet code**: mRNA nucleotides are read in groups of 3 called **codons** in the 5'→3' direction. The start signal is called the **start codon** and is the sequence AUG. This start codon is the reference for reading in triplets from that point until a "Stop" signal, called a **stop codon** is reached.

4.3 –HOW DOES TRANSLATION BUILD A SEQUENCE OF AMINO ACIDS?

Once the start codon is recognized, another type of RNA called **tRNA** will bring the corresponding amino acid. For every amino acid there is a matching tRNA bonded to one amino acid. There are 20 different amino acids, so there are also 20 matching tRNAs. A tRNA also has a nucleotide triplet sequence called an **anticodon** that base pairs to the mRNA codon by hydrogen bonding. The start codon codes for the amino acid methionine (Met) which is brought by its tRNA. The next codon will be read and the matching amino acid will be brought by its matching tRNA. The second tRNA anticodon will base pair with the codon long enough for the ribosome (made of catalytic **rRNA** & proteins) to link the 2 amino acids by a **peptide bond**. This process will repeat & the amino acid sequence will form a long chain until the stop codon is reached. Draw figure 17.14 on p.337 of translation.

4.4 – HOW IS A PROTEIN COMPLETED & TARGETED FOR ITS PROPER LOCATION?

Once the stop codon is reached, the primary amino acid sequence is complete but will undergo further modifications based on its function. Proteins functioning in the cytoplasm such as enzymes and motor proteins, will fold in a complex form (see pp.82-83) with help of special protein folding molecules called **chaperonins** (see p.85). The mature protein will be released and begin its function. These cytoplasm proteins will be translated by ribosomes floating freely in the cytoplasm.

Other proteins will function in the membrane or be exported from the cell to function in other locations. Some examples are the channel and carrier proteins & proteins found in secreted fluids like saliva & intestinal fluids. These proteins require chaperonin folding but also specific transporting mechanisms and "ID tags" to help them be targeted for their destination. Ribosomes in the Rough ER are where these proteins will be translated and receive molecular tags like small carbohydrates. The Rough ER then secretes the completed protein into a vesicle for transport to the Golgi. Within the Golgi, further chemical modifications will be attached to the protein that mark it for its final destination. The Golgi will secrete the protein within a vesicle where it will travel to the cell membrane.

SECTION 5 – DNA MANIPULATION TECHNIQUES

5.1 – HOW DO WE EXTRACT & MANIPULATE GENETIC DATA?

Extraction techniques depend on the sample, but most cells contain the entire genome so most cells can be used to extract DNA from. The cell must be lysed for the DNA to be extracted and then the DNA must be purified so that no other molecules are associated with it. DNA is also very long so trying to analyze the entire genome is cumbersome. Instead, we can use stains or dyes that will bind to specific regions if we are concerned with finding a particular gene and we know where it is. More often though, DNA is cut into fragments by **restriction enzymes** that have specific sequences they will bind to & excise, which tend to occur numerous times throughout a chromosome's sequence. Once completed, there will be many fragments of different sizes. Both the number of fragments & the length of the fragments are unique to an individual since the restriction enzymes are excising DNA sequences at specific points, and no individual has the exact same DNA sequence as another individual.

5.2 – HOW DOES GEL ELECTROPHORESIS WORK?

The DNA fragments are small molecules so we cannot visualize them without some further visualization procedure. **Gel electrophoresis** is a technique that separates molecule fragments based on their size & charge, and finally a stain is applied to visualize their patterns of separation. In DNA electrophoresis, the DNA fragments are loaded into pre-formed cavities within a gel called agarose. A liquid is poured over top of the gel within a chamber and then an electric current is applied that will force molecules to move. DNA is negatively charged (from the phosphate group) so if a negative current is applied right near its fragment cavities, DNA will begin moving away through the porous gel towards the end with a positive current attracting it. Small DNA fragments will move fast since they will fit through the gel pores easily but large fragments will move slowly. Upon completion, the gel will have columns of DNA "bands" like a bar-code. Draw figure 20.9 on p.405 of this technique.

5.3 – HOW DOES PCR WORK?

Polymerase Chain Reaction (PCR) is a technique that allows for producing larger quantities of a DNA sample. For example if only a small sample of blood is found at a crime scene, PCR can replicate it artificially to provide more of that same DNA. This technique is only possible thanks to the discovery of a thermophilic (heat-loving) bacteria that we have extracted a thermophilic DNA polymerase from. To copy DNA quickly and artificially, the temperature must be raised high enough to break the hydrogen bonds between a DNA double helix. The temperature is then dropped to allow the DNA polymerase to begin replication. This heating-cooling cycle will be repeated many times until billions of the same sequence have been generated, meaning a large enough sample to analyze.

5.4 – HOW DOES A NUCLEIC ACID PROBE WORK?

If we know the sequence or part of the sequence of a gene of interest, we can construct a nucleic acid probe. **Nucleic acid probes** are short single-stranded nucleic acid sequences that will hydrogen bond (**hybridize**) with a single stranded DNA where we want to locate a gene of interest. The double-stranded DNA with the gene of interest has already been denatured so its hydrogen bonds have been broken to give 2 single DNA strands.

SECTION 6 – GENETIC ENGINEERING

6.1 – HOW IS GENETIC ENGINEERING DIFFERENT FROM BIOTECHNOLOGY?

Biotechnology refers to methods using genetic information to better our understanding of organisms. **Genetic engineering** is the purposeful manipulation of organism's genomes to generate products we desire. While most genetic engineering is done with good intentions, there will always be unforeseen impacts and you should consider these throughout the trials discussed below.

6.2 – WHAT WAYS HAVE GENETICALLY ALTERED BACTERIA BENEFITED US?

Bacteria reproduce extremely fast so they are ideal organisms to perform genetic experiments with. **Plasmids** are circular pieces of DNA in bacteria that serve various functions to them. They exchange these plasmids with each other and is one reason they mutate so rapidly. We have used these plasmids to insert genes we want to replicate such as insulin genes to produce working copies of insulin genes. We have also been able to study artificially **transforming** bacteria with various genetically modified plasmids, thus giving them novel features like glowing in the dark by inserting the gene from bioluminescent bacteria. A more useful application has been transforming some bacteria to have the ability to consume oil, which has been used a **bioremediation** method to clean up oil spills.

6.3 – WHAT IS THE SIGNIFICANCE OF GM FOODS?

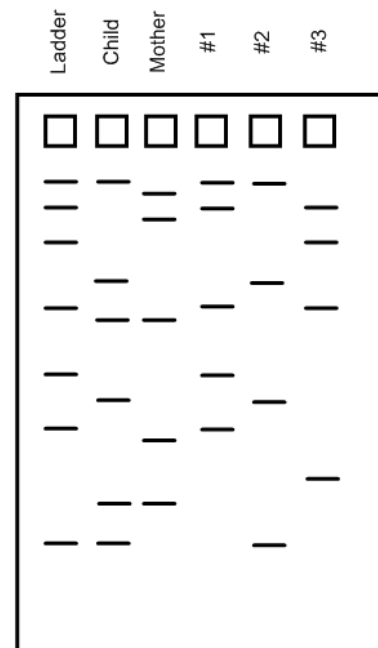
The benefits of **Genetically Modified (GM) foods** are highly debatable. While the methods producing them range from exposure to chemicals to inserting altered genes, it is important to consider their risk-benefit factors. Is a seedless watermelon worth exposure to leftover radiation? Are larger, pathogen-resistant crops worth the potentially insurmountable resistance of the pathogens? Whatever your stance on consumption of these products is, just remember there is always unforeseen risk when we tinker with the genetics of organisms that “nature” does slowly over time.

6.4 – HOW ARE DISEASES & DISORDERS FOUGHT BY GENETIC ENGINEERING?

If we know the gene sequence underlying a disorder, an engineered gene can be inserted & replace the faulty gene in this process of gene therapy. What about cancers? Perhaps the future will hold a method of reproducing your own cells that are genetically modified to be resistant to cancer. A largely debated topic is using stem cells for therapy. **Stem cells** are those that can differentiate into some or any cell based on their point in embryo development. Obviously the source itself is of concern but they may be a potential source of cell regeneration in the future. Imagine being able to use some of your very own stem cells to make “insurance” organs or cells for yourself...would you do it?

1. You are studying an organism and discovered that its genetic information is stored in a circular chromosome. This indicates
 - a. It is a transgenic organism
 - b. It uses RNA to store genetic material
 - c. It is a prokaryote
 - d. Its chromosome is single-stranded
2. Cytosine and guanine always pair in DNA because
 - a. They are both purines, having a double-ring structure
 - b. They form disulfide bonds, unlike adenine – thymine pairs
 - c. They are only present in DNA, not RNA
 - d. They form 3 hydrogen bonds, while adenine – thymine form 2 hydrogen bonds
3. Which of the following is **not** a shared characteristic of both DNA and RNA?
 - a. Uracil bases
 - b. Sugar – phosphate backbones
 - c. Purine – pyrimidine base pairing
 - d. 5' to 3' synthesis
4. A toxin is introduced into an organism which disrupts the three-dimensional configuration of DNA Polymerase. Which of the following would still occur?
 - a. Replication of the leading DNA strand
 - b. Replication of the lagging DNA strand
 - c. Transcription of the DNA
 - d. 2 of the above processes
 - e. All of the above processes
5. In the Meselson & Stahl Experiment, a light isotope & a heavy isotope was used to confirm the semiconservative model of DNA replication. Which of the following is the most accurate concerning their procedures.
 - a. A nitrogen isotope was best since DNA and not RNA was being studied.
 - b. The 2 isotopes of phosphorous helped determine the replication pattern.
 - c. The heavy nitrogen was able to be differentiated from the light nitrogen.
 - d. The isotope forms allowed the frequency of base pairs to be determined in each replication cycle.

6. All of the following statements are true regarding prokaryotic and eukaryotic chromosomes except
- Both prokaryotic and eukaryotic chromosomes are double-stranded
 - Eukaryotic chromosomes are located in the nucleus of the cell, while prokaryotic chromosomes are located in the nucleoid region of the cell
 - Prokaryotic chromosomes undergo replication prior to cell division, eukaryotic chromosomes undergo replication during the mitotic phase
 - Both prokaryotic and eukaryotic organisms utilize DNA polymerase to replicate their chromosomes
7. Which of the following statements regarding translation is false?
- Translation occurs in the cytoplasm of the cell
 - The start codon codes for the amino acid methionine
 - The anticodons of tRNA base pair with the codons of mRNA at the ribosome
 - Codons are pairs of nucleotides in a RNA strand
8. A small amount of DNA is found at a crime scene. To increase the availability of this DNA for testing technicians should use
- Gel electrophoresis
 - Polymerase chain reaction
 - Restriction enzymes
 - Bioengineering
9. A gel is run using the DNA of a child, his mother, and three potential fathers. Using the results to the right, determine who the father of the child is.
- Man #1
 - Man #2
 - Man #3
 - None of the above



10. Bacteria can be used to synthesize human insulin for diabetic patients. Which of the following correctly illustrates the steps necessary to produce this medication?
- Cut the gene from the human genome using restriction enzymes, amplify the amount of DNA using gel electrophoresis, insert the gene on a plasmid, transform bacteria using the plasmid
 - Cut the gene from the human genome using restriction enzymes, amplify the amount of DNA using polymerase chain reaction, insert the gene into bacteria, incubate the bacteria at 37°C
 - Locate the gene using a DNA sequencer, remove the gene from the genome, amplify the amount of DNA using polymerase chain reaction, insert the gene onto a plasmid, transform bacteria using the plasmid
 - Locate the gene using a DNA sequencer, remove the gene from the genome, amplify the amount of DNA using polymerase chain reaction, insert the gene onto a plasmid, insert the plasmid into human cell

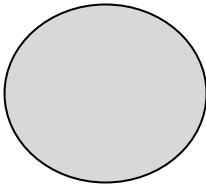
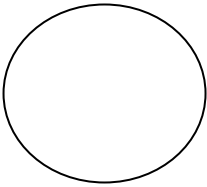
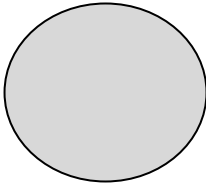
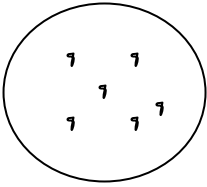
Template strand: 5' T A C A A A G G C G T A A T T 3'

11. The primary sequence of the protein which results from the above strand of DNA is
- Tyr – Lys – Gly – Val – Ile
 - Asn – Tyr – Ala – Phe – Val
 - Met – Phe – Gly – His – Stop
 - Leu – Met – Arg – Lys – His
12. The protein coding mRNA of eukaryotes is generally different in length than the DNA coding for it. In prokaryotes however, the DNA is usually similar in size to the transcribed mRNA. Which of the following best explains this finding?
- Prokaryotes contain exons while eukaryotes contain introns.
 - Prokaryotes lack ribosomes to translate their proteins the same as eukaryotes.
 - Prokaryotes lack spliceosomes.
 - Eukaryotes have more ribosomes than prokaryotes.

Questions 13 & 14

In a transformation experiment, a sample of *E. coli* bacteria was mixed with a plasmid containing the gene for resistance to the antibiotic ampicillin (amp^r). Plasmid was not added to a second sample. samples were plated on nutrient agar plates, some of which were supplemented with the antibiotic ampicillin. The results of the *E. coli* growth are summarized below. The shaded area represents extensive growth of bacteria; dots represent individual colonies of bacteria.

NUTRIENT AGAR PLATES

	No Ampicillin	Ampicillin
Wild-type <i>E. coli</i>	I 	II 
<i>E. coli</i> and amp^r plasmid	III 	IV 

13. Plates I and III were included in the experimental design in order to
- demonstrate that the *E. coli* cultures were viable.
 - demonstrate that the plasmid can lose its amp^r gene.
 - demonstrate that the plasmid is needed for *E. coli* growth.
 - prepare the *E. coli* for transformation.
14. In a second experiment, the plasmid contained the gene for human insulin as well as the amp^r gene. Which of the following plates would have the highest percentage of bacteria that are expected to produce insulin?
- I only
 - III only
 - IV only
 - I and III

Part 2: Free Response

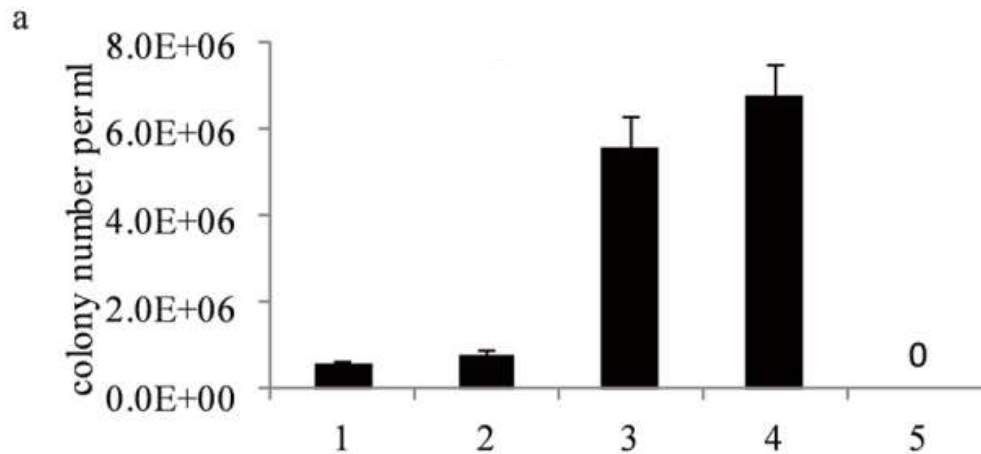


Figure a: The effect of 5 different temperatures (symbolized by 1-5) on bacterial transformation efficiency. Note: The numbers 1-5 do not equal the temperatures.

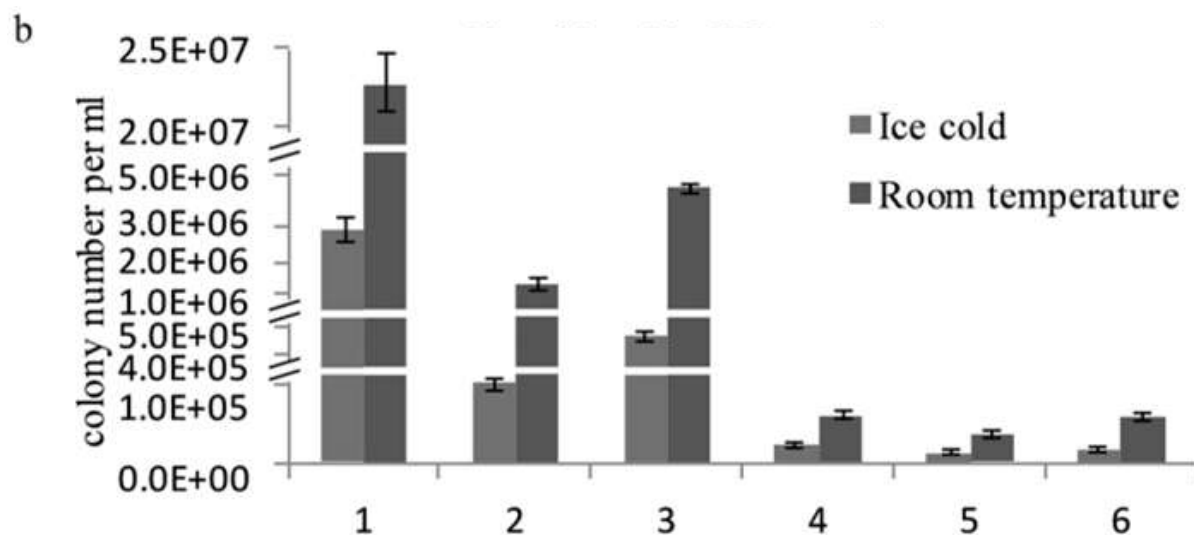


Figure b: The effect of 6 different plasmids (symbolized by 1-6) on bacterial transformation efficiency under 2 different temperatures.

1. Refer to the figures above to answer the prompts below.

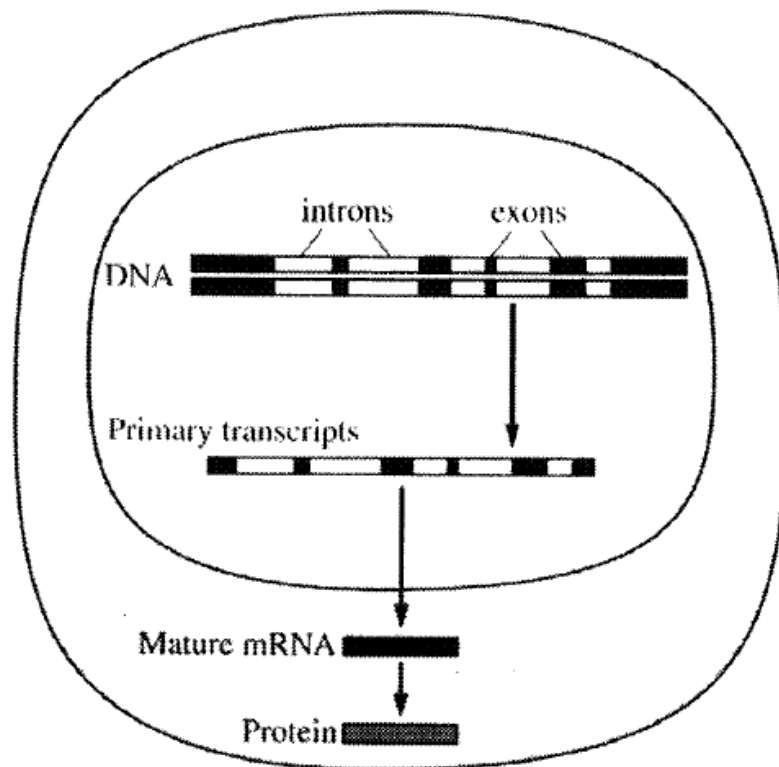
- Calculate** how many more times effectiveness the best temperature treatment has compared to the least effective temperature treatment.
- State a null hypothesis** that would be best tested using the data in figure b.
- Explain** whether to accept or reject your null hypothesis based on the data shown in figure b.

2.

In 1952 Alfred Hershey and Martha Chase used radioactive phosphorus and radioactive sulfur to selectively label the DNA and proteins of bacteriophage T2, a virus that infects bacteria. After incubating the labeled bacteriophage particles with *Escherichia coli* and separating extracellular phage particles from the bacteria, Hershey and Chase measured the amounts of radioactive phosphorus and sulfur inside infected *E. coli* cells and in the liquid growth medium outside the *E. coli* cells.

- (a) **Predict** the experimental result that would best support the claim that DNA is the source of heritable information, and **provide** reasoning to explain how the result supports the claim.
- (b) Bacteriophages, like other viruses, consist primarily of a protein coat and packaged DNA. **Describe** the function of ONE critical enzyme in bacterial cells that is necessary for replicating bacteriophage DNA.

3.



The figure represents the process of expression of gene X in a eukaryotic cell.

- (a) The primary transcript in the figure is 15 kilobases (kb) long, but the mature mRNA is 7 kb in length. **Describe** the modification that most likely resulted in the 8 kb difference in length of the mature mRNA molecule. **Identify** in your response the location in the cell where the change occurs.
- (b) **Predict** the length of the mature gene X mRNA if the full-length gene is introduced and expressed in prokaryotic cells. **Justify** your prediction.

4.

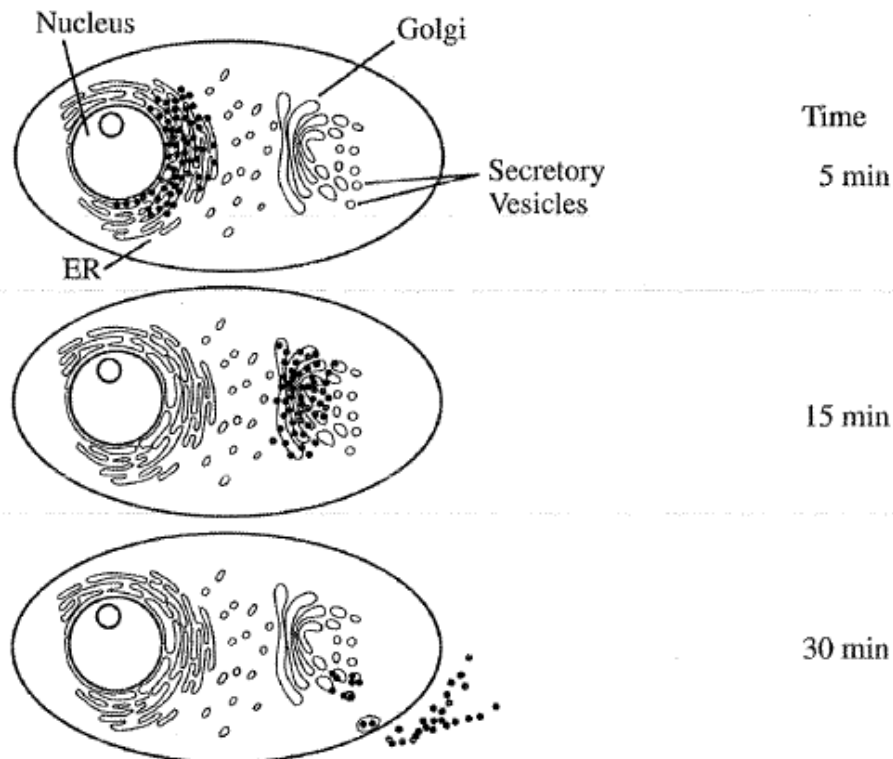


Figure 1. Radioactively labeled polypeptides

In an experiment investigating the mechanism of protein secretion, researchers tracked the movement of radioactively labeled polypeptides in pancreatic cells. At various times after the labeling, samples of the cells were observed using an electron microscope to determine the location of the radioactively labeled polypeptides. Figure 1 summarizes the results at 5 minutes, 15 minutes, and 30 minutes. The dark dots in the figure represent the radioactively labeled polypeptides.

- Using the experimental results, **describe** the pathway that secretory proteins take from their synthesis to their release from the cell.
- Predict** what the results would be if mRNA were radioactively labeled instead of polypeptides. **Give reasoning** to support your prediction.